ECOPHYSIOLOGY

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Modulation of relative growth rate and its components by water stress in Mediterranean species with different growth forms

Abstract Effects of water availability on seedling growth were analysed in eight Mediterranean species naturally occurring in the Balearic Islands. Seedlings were grown outdoors during summer under two irrigation treatments: field capacity and 35% of field capacity. The relative growth rate (RGR) strongly depended on the growth form, from highest values in herbs to lowest in woody perennials. The main component associated with interspecific variation in RGR was the specific leaf area (SLA), and a quantitative grouping of the different growth forms appeared along the regression line between both parameters. The slow-growing species, i.e. woody perennial shrubs, had the lowest SLA and the fast-growing perennial herbs, the highest, while woody semi-deciduous shrubs appeared intermediate. Decreases in RGR due to water stress were analysed in terms of the relative contribution of the leaf mass ratio (LMR), SLA and the net assimilation rate (NAR). Pooling all species, the decrease in RGR caused by water deficit was mainly explained by decreases in SLA. However, this general pattern was strongly dependent of growth form. Thus, in the woody perennial plants, the decrease in RGR was accompanied by a three-fold decrease in NAR which, however, increased in perennial herbs. SLA increased with decreasing water supply in woody perennial plants, and decreased in woody semi-deciduous shrubs and perennial herbs. Finally, decreases in LMR partly explained decreases in RGR in perennial herbs and woody perennial shrubs. This different response of the different growth forms may reflect differences in seedling adaptation and surviving strategies to drought periods.

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Introduction

The Mediterranean climate is characterised by a hot dry period in summer and a cool wet period in winter. From an ecophysiological point of view, the variability and unpredictability of precipitation imposes strong constraints on plants that could be extremely important for the survival of individuals (Joffre et al. 1999). In particular, the effects of water deficits during the summer severely influence the distribution and composition of vegetation in the Mediterranean basin. Moreover, the global change effects on the Mediterranean climate likely provide more frequent and longer drought periods (Osborne et al. 2000).

It is well known that seedling establishment is a critical developmental stage that may strongly depend on water availability (Moles and Westoby 2004). Plant relative growth rate (RGR) can be factorised into physiological and morphological components that determine the plant's carbon economy (Lambers et al. 1989). The physiological component is the net assimilation rate (NAR) and it is a measure of whole-plant daily net rate of change in plant carbon content (McKenna and Shipley 1999; Poorter 1989). NAR is generally correlated with the rate of photosynthesis per unit leaf area (Konings 1989; Poorter and Van der Werf 1998). The morphological component is related to the amount of leaf area per plant mass or the leaf area ratio (LAR), which, in turn, depends on two components: specific leaf area (SLA) and a measure of biomass allocation (leaf mass ratio, LMR) (Konings 1989; Poorter and Van der Werf 1998). The interspecific variation in seedling RGR is often strongly correlated with SLA in a

large number of studies spanning a wide range of growth forms, originating from many habitats (Lambers and Poorter 1992; Poorter and Van der Werf 1998; Reich et al. 1998; Wright and Westoby 1999; Wright and Westoby 2000). By contrast no generality has emerged for the relationships between RGR and LMR or between RGR and NAR (Lambers and Poorter 1992; Poorter and Van der Werf 1998). However, Shipley (2002) stated that the relative importance of SLA and NAR changes depending on irradiance, proposing a trade-off between these components as a function of daily irradiance.

Differences in RGR and its components among species and functional groups have been related to different adaptation patterns to the environment (Lambers and Poorter 1992). For instance, early-successional species, as well as annual species, tend to be characterised by high RGRs, while late-successional species and perennials have, in general, a lower RGRs (Poorter and Garnier 1999). Even within a single functional group, differences in RGR due to e.g. a different evolutionary history may lead to differences in competitiveness. In Hawaii, for instance, it has been shown that native species have a lower photosynthetic capacity, SLA and RGR than invasive species, and this has been related to the competitive and invasive ability of these species (Baruch and Goldstein 1999; Durand and Goldstein 2001; Pattison et al. 1998).

Water stress must be a major limiting factor for RGR in semi-arid climates, such as the Mediterranean, strongly constraining both growth and seedling survival during late spring and summer (Moles and Westoby 2004; Volaire et al. 1998). Despite its importance, the number of analyses of the effects of water stress on RGR is surprisingly scarce, and limited to very few species, notably grasses (Bargali and Tewari 2004; Kalapos et al. 1996; Retuerto and Woodward 1993; Van den Boogaard et al. 1995, 1996, 1997; Van Splunder et al. 1996; Wang et al. 1998). Traits such as greater allocation of biomass below than above ground, a lower evaporative surface, and a higher leaf mass per unit leaf area are common water-stress effects on biomass allocation (Ludlow 1989). However, very few authors have factorised the causes of the variation in RGR due to water stress. Poorter and Nagel (2000), after reviewing the limited published data, attributed the decrease in RGR due to water stress to a decrease in NAR, and, to a lesser extent, to a decrease in SLA. However, Van den Boogaard et al. (1997) did not find significant differences in the contribution of LAR and NAR to variation in RGR between well-watered and water-stressed wheat cultivars. Similarly, Ball and Pidsley (1995) and Ball (2002) showed in two mangrove species that RGR decline with increase in salinity was due to decrease in both NAR and LAR. As far as we know, there is no comparative study including several species comprising different growth forms in which growth of plants at limiting water supply has been analysed in terms of RGR and its components. Moreover, the different growth forms (i.e. perennials versus semi-deciduous and herbs versus woody species) differ in their morphological and physiological responses to water stress (Flexas et al. 2003; Ludlow 1989). This makes it reasonable to hypothesise different strategies in the effects of drought on the relative contribution of NAR, SLA and LMR to the decrease in RGR across species comprising different growth forms. For instance, for species with a rapid leaf turnover such as deciduous plants and herbs, adjustments of RGR through changes in SLA and LMR in response to drought are likely to occur. By contrast, for woody perennial species changes associated with morphological adjustments may be expected only at early stages of development, while in adult plants most changes may be expected to correlate with adjustments in NAR.

In this report, we studied the effects of water stress on seedling establishment of eight Mediterranean species comprising four growth forms. The objective of the present work was to determine the relative contribution of each of the underlying growth parameters to the decrease in RGR caused by water deficit across species comprising different growth forms.

Materials and methods

Plant material, environmental conditions and treatments

Eight Mediterranean species including different growth forms and occurring in different habitats were selected for this study (Table 1). Species were classified depending on their growth form into: perennial herbs (PH), woody perennial shrubs (WPS), woody semi-deciduous shrubs (WSS) and woody perennial plants (WPP).

Plant material was obtained from seeds collected from natural populations of the selected species (Table 1). The smallest and the biggest seeds of those species showing high variability in the seed weight were discarded to homogenise initial seedling weight and increase the reliability of RGR estimates (Poorter and Garnier 1996).

Seeds were germinated on filter paper moistened with deionized water in a controlled environment (germination chamber, at 18°C in darkness). The experiment was performed in the first week of May 2002 for *Phlomis italica* (PI), *Cistus albidus* (CA), *Limonium magallufianum* (LM) and *Limonium gibertii* (LG), or 2003 for *Hypericum balearicum* (HB), *Pistacia lentiscus* (PL), *Beta vulgaris* subsp. *marcosii* (MC), and *Beta vulgaris* subsp. *maritima* (MT). On the day following radicle emergence, seedlings were planted individually in pots (20-cm height, 4.1 l volume) containing a 40:40:20 mixture of clay-calcareous soil, horticultural substrate and perlite. For each species, seedlings of similar initial size were assigned to each treatment. Seedlings of each species and treatment were then randomly distributed under a shade cloth with a 30% light exclusion outdoors at the

Table 1 Species analysed and their characteristics

Species	Code	Family	Seed sources	Description
Beta maritima L. subsp. marcosii A. Juan & M. B. Crespo	MC	Chenopodiaceae	Ses Bledes N 39°08'19"E 02°57'41"	Perennial herb. Endemic of the Balearic Islands, inhabits in few very small islets subjected to saline spray and with high nutrient inputs
Beta maritima L. subsp. maritima	MT	Chenopodiaceae	Cap Salines N 39°15'58"E 03°03'02"	Perennial herb inhabiting coastal ecosystems. Widespread in temperate climates
Phlomis italica L.	PI	Labiatae	Artà N 39°44'44"E 03°20'55"	Semi-deciduous shrub up to 1 m, densely covered by hairs. Endemic of the Balearic Islands. The biggest populations are found 500 m above the sea level, where they compete with CA
Cistus albidus L.	CA	Cistaceae	Artà N 39°43'58"E 03°21'57"	Semi-deciduous shrub up to 1 m. Commonly found in the Mediterranean shrubland. As PI, their leaves are covered by hairs
Limonium magallufianum L. Llorens	LM	Plumbaginaceae	Magalluf N 39°30'22"E 02°32'46"	Woody perennial plant, in cushion-like rosettes. Endemic of the Balearic Islands, inhabiting just in one coastal marsh located in Mallorca
Limonium gibertii (Sennen) Sennen	LG	Plumbaginaceae	Es Carnatge N 39°32'39"E 02°41'50"	Woody perennial plant, in cushion-like rosettes. Occurring in West Mediterranean rocky and sandy coastal areas
Hypericum balearicum L.	HB	Guttiferae	Mortitx N 39°52'25"E 02°55'10"	Perennial shrub up to 2 m, endemic of the Balearic Islands. The biggest populations are found in the shrubland 500 m above the sea level, where competes with PL
Pistacia lentiscus L.	PL	Anacardiaceae	Mortitx N 39°52'25"E 02°55'10"	Perennial shrub up to 5 m, commonly found in the Mediterranean shrubland

University of the Balearic Islands (Mallorca, Spain). Eventual instantaneous light measurements were performed to ensure that all seedlings were receiving equal amounts of irradiance.

The environmental conditions during the experiment were characteristic for the typical late spring-summer Mediterranean climate, with high temperatures and low relative humidity which generate considerable water losses by evapotranspiration. The maximum average temperature during the experiment in 2002 was recorded in July (24.1°C), and the minimum in May (18.1°C). 2003 was a somewhat hotter year, with a maximum average temperature of 27.7°C in August and a minimum of 18.6°C in May. By contrast, total irradiance during the experiment was similar between both years (2,517 and 2,674 MJ m^{-2}). Consequently, the mean monthly evapotranspiration was about 160 l m^{-2} in both years.

The transplanted seedlings were equally well watered during the first 40 days prior to exposure to water stress. In their habitats, seeds germinate and seedlings establish in early spring when soil moisture is adequate before experiencing water stress in summer. After that, seedlings were randomly assigned to two irrigation treatments: (a) plants maintained at field capacity (control), and (b) plants maintained at soil water deficit (35% of field capacity). Desired moisture levels were attained by allowing the soil to dry until close to the selected moisture level, as determined gravimetrically on each pot. The pots were weighed on alternate days, and the required amount of water was added in order to maintain the correct moisture level.

Growth parameters

In their habitats, these species typically endure 3–5 months of water stress. Therefore, growth of seedlings was monitored by harvesting six plants per species and treatment, 40 (H1), 80 (H2), 120 (H3) and 160 days (H4) after germination. Seedling mortality prior to the onset of the experiment obliged to reduce the number of replicates to four in two of the species, *P. lentiscus* and *H. balearicum*. After harvesting, total plant leaf area (LA, m^2) was determined using an AM-100 leaf area meter (ADC, Herts, UK) and roots, stems and leaves were separated and dried in a ventilated oven at 60°C until constant weight was reached. From these components, stem dry matter (SDM; g), leaves dry matter (LDM; g) and root dry matter (RDM; g) were determined. Based on these data, the following plant traits were calculated: total plant biomass (B; g), specific leaf area (SLA; m^2 kg^{-1}), leaf mass ratio (LMR; g g^{-1}), leaf area ratio (LAR; m^2 kg^{-1}), stem mass ratio (SMR; g g^{-1}) and root mass ratio (RMR; g g^{-1}). Average data from each species and treatment for two consecutive samplings were used to compute the partial net assimilation rate (NARp; g m^{-2} day^{-1}) and the partial relative growth rate (RGRp; mg g^{-1} day^{-1}) by the following equations:

$$NAR = \frac{(B_2 - B_1) \times (\log_e LA_2 - \log_e LA_1)}{(t_2 - t_1) \times (LA_2 - LA_1)}$$

$$RGR = \frac{\log_e B_2 - \log_e B_1}{t_2 - t_1}$$

where 1 and 2 refer to two consecutive harvests. The total NAR (NARt) and total RGR (RGRt) for the entire study period were calculated using the same formulae with t_1 and t_4. Leaf-related variables were calculated excluding the petiole weight.

Effects of growth form, species and moisture level on seedling growth parameters were analysed by one-way ANOVA. All statistical analyses were performed using the SPSS package (SPSS, v10.0). To determine the change in each of the growth parameters (NARt, SLA and LMR), scaled with respect to the relative change in RGRt, we calculated the growth response coefficient (GRC) according to Poorter and Nagel (2000).

Results

Table 2 shows mean values of total seedling biomass (B), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), leaf area (LA) and derived growth parameters. Both under control and water-stress conditions, the two *Beta* species were the species with the highest LA and growth rates. On the other hand, the perennial shrubs HB and PL had the lowest. As common patterns, all species increased LA and reduced SLA with ageing, but their LAR and LMR decreased. Also, they tended to increase RMR with age. Apart from these general trends, the response of the remaining parameters with increase in plant age was strongly species-dependent. Most of them showed their highest RGRp and NARp at early stages of development, but others, especially, the perennial shrubs, increased both parameters in the third harvest. These temporal variations in RGRp were mainly explained by changes in NARp, according to the strong correlation between both parameters (r ranged from 0.833 in PI to 0.993 in MC). Regarding to the effects of drought on these parameters, the most distinctive feature was the strong difference between growth forms (Table 2). For instance, even under water stress, perennial herbs had approximately a ten-fold greater LA than non-stressed perennial shrubs in the fourth harvest.

Figure 1 shows the evolution of plant biomass under well-watered and water-stressed conditions and the evolution of the percentage of plant biomass under water-stressed with respect to the control values. The two *Beta* species were the species with the highest biomass at all harvests, both under well-watered and water-stressed conditions, while the woody perennial shrubs HB and PL had the lowest values. At the end of the experiment, the percentage of plant biomass under water-stressed with respect to the control values ranged between 30% and 60% for all species. However, that of woody perennial plants (LM and LG) and shrubs (HB and PL) was higher than 50%, while that of the remaining species was lower than 50%. For most of the species (MC, MT, LG, HB and PL) the effects of water stress on biomass reduction were more notorious early in the experiment, with strong reductions in plant biomass from H1 to H2. However, for PI, CA and LM the percentages of plant biomass under water-stressed with respect to the control values at H2 were still 90, 116 and 88%, respectively.

The highest RGRt both in control and stressed plants was that of MC (61 ± 1 and 52 ± 2 mg g^{-1} day^{-1}, respectively), while the lowest was that of PL (35 ± 2 and 31 ± 1 mg g^{-1} day^{-1}) (Fig. 2a). CA had the highest reduction in RGRt due to water stress (23%) and LG the lowest (8%). Regarding growth form, perennial herbs had the highest RGRt, with 57 ± 3 and 50 ± 3 mg g^{-1} day^{-1}, under control and water stress, respectively (Fig. 2b). Perennial shrubs had the lowest, 40 ± 5 and 36 ± 5 mg g^{-1} day^{-1}. The mean RGRt for all species together was 50 ± 3 mg g^{-1} day^{-1} in non-stressed plants and 43 ± 2 mg g^{-1} day^{-1} under water stress.

A regression analysis was performed to relate RGRt with each of its components (Fig. 3). ANCOVA revealed non-significant differences in the regression lines between well-watered and water-stressed plants, for which only pooled plots are shown.

The highest RGRt values were found for those species with the smallest NARt (Fig. 3a). From the lowest to the highest NARt and from the highest to the lowest RGRt the following order was obtained: perennial shrubs, semi-deciduous shrubs, woody perennial plants and perennial herbs. However, plotting together data of the final harvest from all species and treatments a non-significant relationship between NARt and RGRt was obtained ($r = -0.466$, $P > 0.05$) (Fig. 3a). By contrast, when plotting each species separately, including all harvests and both treatments (well watered and water stress), a significant, but positive relationship between RGRp and NAR was obtained for all species ($P < 0.01$) (data not shown).

The global positive relationship between final LAR and RGRt was found to be highly significant ($r = 0.726$, $P < 0.001$) (Fig. 3b) and, consequently, the relationships between LMR and SLA and RGRt were significant as well ($r = 0.541$, $P < 0.05$ and 0.715, $P < 0.01$, respectively) (Fig. 3c, d). Although it was not possible to establish statistically different groups of species, the four growth forms appeared again quantitatively in different regions of the relationship RGRt–SLA, with the same order than in Fig. 3a. *Limonium* species likely have a higher RGRt at a fixed SLA, pointing to a higher growth capacity. In all species but the two *Limonium*, SLA decreased when RGRt decreased due to water stress. When plotting each species separately, including all harvests and both treatments (well-watered and water-stress), also a significant, positive relationship between RGRp and LAR was obtained for all species ($P < 0.01$), except for *H. balearicum* and *P. lentiscus* (data not shown).

Table 2 Mean values of total seedling biomass (B), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), partial relative growth rate (RGRp) and partial net assimilation rate (NARp) for each species, treatment and harvest

Species	Treatment	Harvest	B (g)	LMR	SMR	RMR	LA (m^2)	SLA ($m^2\ kg^{-1}$)	LAR ($m^2\ kg^{-1}$)	RGRp ($mg\ g^{-1}\ day^{-1}$)	NARp ($g\ m^{-2}\ day^{-1}$)
MC	Control	H1	0.01	0.65	0.06	0.28	0.03	46.2	29.7		
MC	Control	H2	2.28	0.66	0.06	0.28	4.62	31.8	21.2	137	6.4
MC	Water-stressed	H2	1.31	0.59	0.07	0.34	2.29	29.8	17.4	124	6.4
MC	Control	H3	8.93	0.55	0.08	0.38	12.85	26.7	14.5	34	2.0
MC	Water-stressed	H3	3.95	0.49	0.11	0.40	3.78	20.0	9.7	27	2.2
MC	Control	H4	12.99	0.54	0.07	0.39	16.70	24.1	13.0	10	0.8
MC	Water-stressed	H4	4.91	0.41	0.11	0.47	3.21	16.1	6.6	6	0.7
MT	Control	H1	0.02	0.79	0.07	0.14	0.06	46.3	36.4		
MT	Control	H2	1.55	0.65	0.06	0.30	2.98	31.0	20.1	112	5.1
MT	Water-stressed	H2	0.96	0.54	0.07	0.38	1.44	27.3	14.8	100	5.3
MT	Control	H3	6.56	0.52	0.10	0.39	8.46	26.0	13.5	36	2.4
MT	Water-stressed	H3	3.92	0.49	0.10	0.40	4.84	25.0	12.3	35	2.6
MT	Control	H4	10.24	0.51	0.07	0.42	12.61	24.7	12.6	11	0.9
MT	Water-stressed	H4	4.93	0.48	0.13	0.39	4.89	21.8	10.3	5	1.7
PI	Control	H1	0.02	0.63	0.10	0.26	0.05	35.2	22.3		
PI	Control	H2	0.41	0.53	0.12	0.34	0.31	13.9	7.4	73	6.7
PI	Water-stressed	H2	0.37	0.48	0.11	0.41	0.23	13.2	6.2	71	7.4
PI	Control	H3	4.58	0.49	0.18	0.32	3.35	14.8	7.3	61	8.3
PI	Water-stressed	H3	2.16	0.45	0.14	0.41	1.00	10.2	4.6	45	8.7
PI	Control	H4	8.58	0.41	0.15	0.44	5.00	14.2	5.9	16	2.6
PI	Water-stressed	H4	3.11	0.41	0.12	0.47	1.38	10.5	4.3	8	1.5
CA	Control	H1	0.02	0.69	0.12	0.18	0.04	37.1	25.8		
CA	Control	H2	0.47	0.41	0.15	0.45	0.47	24.9	10.1	83	6.3
CA	Water-stressed	H2	0.55	0.43	0.12	0.45	0.37	15.2	6.6	86	8.5
CA	Control	H3	2.56	0.44	0.14	0.41	1.52	13.5	6.0	42	5.8
CA	Water-stressed	H3	1.10	0.39	0.11	0.50	0.43	10.1	3.9	19	5.1
CA	Control	H4	5.49	0.29	0.20	0.51	2.32	14.0	4.1	19	3.4
CA	Water-stressed	H4	1.48	0.35	0.19	0.46	0.57	11.0	3.8	7	1.8
LM	Control	H1	0.01	0.79	0.00	0.21	0.03	29.3	22.9		
LM	Control	H2	1.28	0.65	0.05	0.29	1.16	14.1	9.3	117	10.6
LM	Water-stressed	H2	1.13	0.59	0.08	0.33	0.82	11.6	6.8	109	11.0
LM	Control	H3	3.95	0.56	0.11	0.33	1.90	8.6	4.9	29	4.7
LM	Water-stressed	H3	1.69	0.55	0.11	0.34	0.95	10.5	5.8	15	5.2
LM	Control	H4	6.47	0.49	0.06	0.44	2.71	8.5	4.2	12	2.8
LM	Water-stressed	H4	3.24	0.52	0.10	0.38	1.72	10.2	5.3	17	3.0
LG	Control	H1	0.01	0.77	0.00	0.23	0.03	31.3	24.5		
LG	Control	H2	0.68	0.67	0.09	0.24	0.76	17.5	11.9	96	7.2
LG	Water-stressed	H2	0.40	0.69	0.08	0.24	0.49	18.0	12.3	85	5.9
LG	Control	H3	3.86	0.56	0.08	0.36	2.80	13.1	7.3	46	5.4
LG	Water-stressed	H3	2.35	0.58	0.09	0.32	1.83	13.2	7.7	45	4.7
LG	Control	H4	6.58	0.49	0.10	0.41	3.02	9.4	4.6	14	2.8
LG	Water-stressed	H4	3.93	0.51	0.12	0.37	2.08	10.4	5.3	13	3.1
HB	Control	H1	0.01	0.63	0.13	0.24	0.01	23.9	15.1		
HB	Control	H2	0.03	0.56	0.11	0.33	0.02	14.4	8.1	40	3.9
HB	Water-stressed	H2	0.01	0.51	0.08	0.41	0.01	11.1	5.7	26	2.9
HB	Control	H3	0.69	0.36	0.25	0.40	0.29	11.9	4.2	83	16.4
HB	Water-stressed	H3	0.51	0.26	0.30	0.44	0.10	7.5	1.9	90	34.1
HB	Control	H4	1.15	0.30	0.28	0.41	0.35	10.1	3.1	13	3.9
HB	Water-stressed	H4	0.64	0.26	0.29	0.46	0.14	9.1	2.3	5	2.8
PL	Control	H1	0.02	0.75	0.04	0.21	0.03	18.1	13.6		
PL	Control	H2	0.06	0.68	0.17	0.14	0.08	18.0	12.3	22	1.7
PL	Water-stressed	H2	0.04	0.64	0.16	0.21	0.04	17.0	11.0	7	0.2
PL	Control	H3	0.99	0.52	0.26	0.22	0.42	8.2	4.3	71	12.0
PL	Water-stressed	H3	0.61	0.29	0.31	0.39	0.13	6.8	2.0	73	20.3
PL	Control	H4	1.69	0.45	0.31	0.24	0.46	6.1	2.8	14	4.3
PL	Water-stressed	H4	1.01	0.32	0.31	0.36	0.17	5.3	1.7	13	8.9

The RGRp and NARp were calculated from two consecutive harvests. The periods for the different harvests were 40, 80, 120 and 160 days after germination. Drought treatment started after H1. The values given are means of four to six replicates per species, treatment and sampling period

Figure 4a shows GRC for each species. Decreases in RGRt due to water stress in LM and LG were mostly associated with a decrease in NARt ($GRC_{NAR} = 3.4$ and 2.9, respectively), while LMR, and especially SLA, increased. In PI the only factor affected by the water-stress treatment and accounting for the decrease in RGRt was
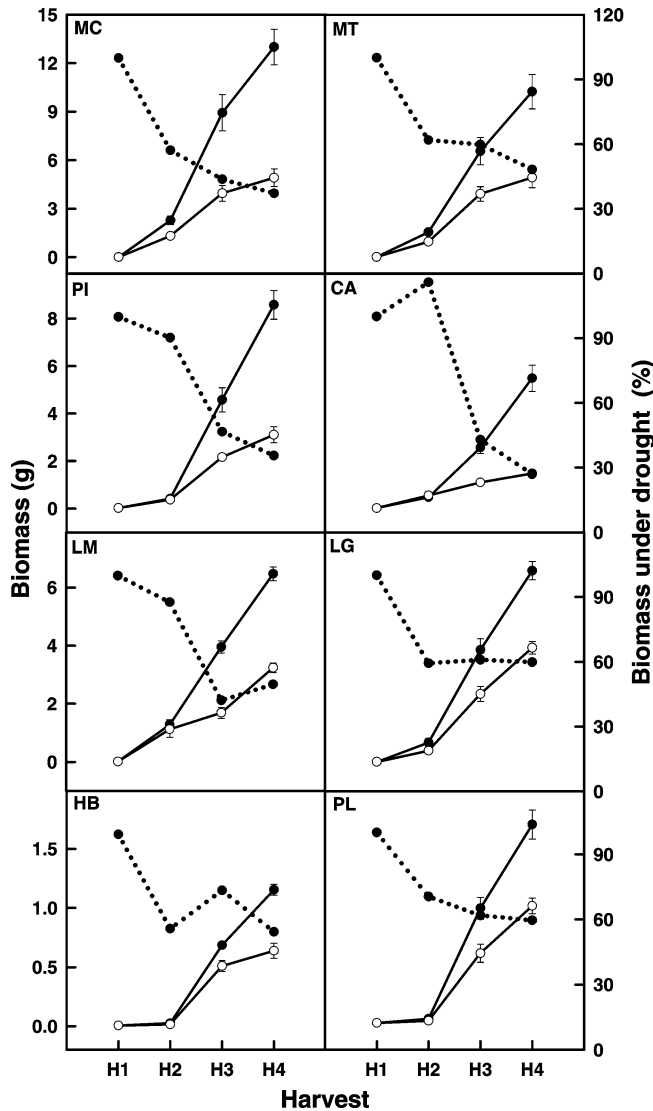
Fig. 1 Biomass evolution along the four harvests under well-watered (*filled symbols*) and water-stress conditions (*empty symbols*) and percentage of biomass under water-stressed conditions with respect to biomass under well-watered conditions (*dotted line*) for each species. Species codes as in Table 1. The values for biomass evolution are means ± standard errors of four to six replicates per species, treatment and sampling period. The values for percentage of biomass under water-stressed conditions with respect to biomass under well-watered conditions are means of four to six replicates per species, treatment and sampling period

Fig. 2 RGRt for each of the species studied (**a**), growth form and pooling all species together (**b**), under well-watered (*black bars*) and water-stress conditions (*grey bars*). Woody semi-deciduous shrubs (*WSS*), woody perennial shrubs (*WPS*), perennial herbs (*PH*) and woody perennial plants (*WPP*). Species codes as in Table 1. In Fig. 2a, the values are means ± standard errors of four to six replicates per species, treatment and sampling period. In Fig. 2b, the values are means ± standard errors for all the species belonging to a particular group

the decrease in SLA ($GRC_{SLA} = 0.84$). However, the other semi-deciduous shrub, CA, also decreased its NARt ($GRC_{NAR} = 0.81$), and therefore increased the LMR ($GRC_{LMR} = -0.53$). HB and PL mainly decreased LMR ($GRC_{LMR} = 0.78$ and 0.95, respectively). The decrease in MT RGRt was mainly associated with decreases in SLA ($GRC_{SLA} = 0.81$), but MC also decreased their LMR ($GRC_{LMR} = 0.93$) and so NAR increased ($GRC_{NAR} = -1.3$).

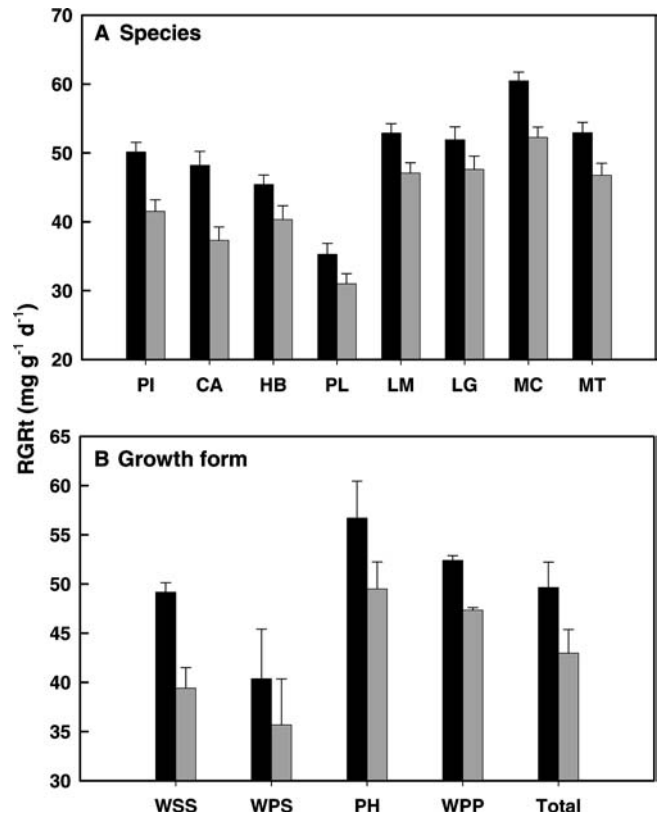Figure 4b shows the relative contribution of each of the underlying growth parameters to the decrease of RGRt due to water deficit across the different growth forms. In woody semi-deciduous shrubs decreases in NARt ($GRC_{NAR} = 0.46$) and especially in SLA ($GRC_{SLA} = 0.74$) mostly explained the decrease in RGRt. In woody perennial shrubs, RGRt decreased due to water stress mainly because of decreases in LMR ($GRC_{LMR} = 0.9$). Perennial herbs strongly increased the physiological component ($GRC_{NAR} = -1.28$), and, thus, the morphological component was strongly decreased and responsible for the overall decrease in RGRt ($GRC_{SLA} = 1.39$ and $GRC_{LMR} = 0.88$). On the other hand, important decreases in NARt ($GRC_{NAR} = 3.13$), partially compensated by increases in SLA ($GRC_{SLA} = -1.54$) and in LMR ($GRC_{LMR} = -0.59$), were associated with the decreases in RGRt due to water stress in woody perennial plants.

Considering all species together, decreases in RGRt were accounted for by decreases in all three components underlying growth ($GRC_{SLA} = 0.59$, $GRC_{LMR} = 0.24$ and $GRC_{NAR} = 0.17$) (Fig. 4b).

Fig. 3 Relationship between RGRt and NARt (**a**) and NAR (**b**), LMR (**c**) and SLA (**d**) at the end of the experiment for the species analysed under well-watered (*filled symbols*) and water-stress conditions (*empty symbols*). Woody semi-deciduous shrubs (*WSS*), woody perennial shrubs (*WPS*), perennial herbs (*PH*) and woody perennial plants (*WPP*). Species codes as in Table 1. The values are means of four to six replicates per species, treatment and sampling period
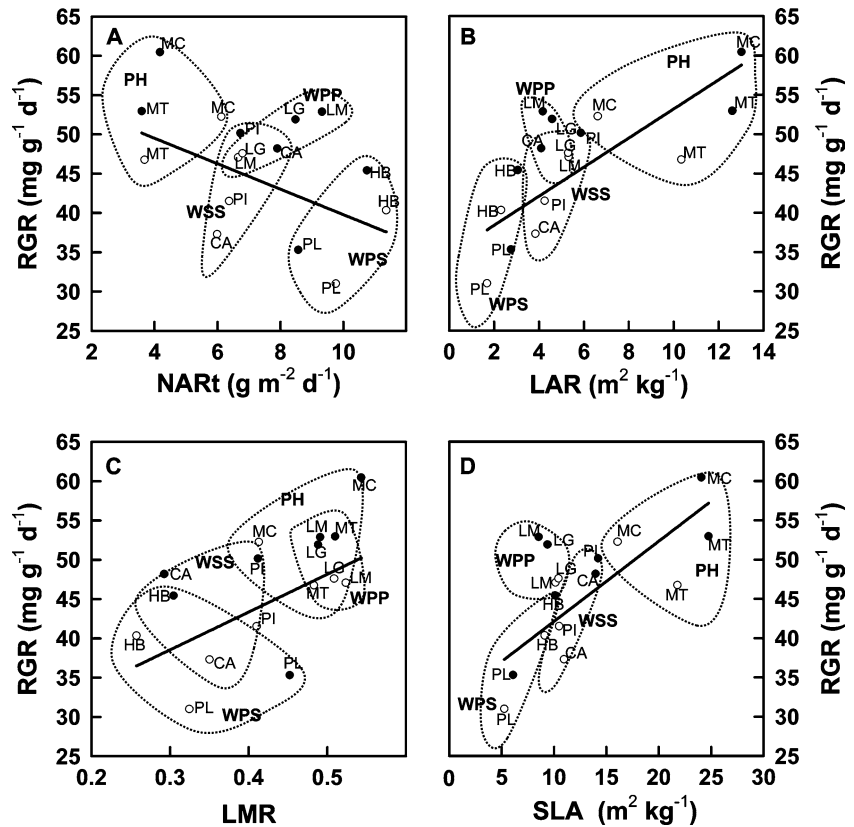
Discussion

Despite the fact that the most widespread soil-related stresses are nutrient shortage and drought (Poorter 2002), only few studies, involving a limited number of species, have been published analysing the causes of variation in RGR as affected by water availability (Poorter and Nagel 2000). In this context, the main contribution of this study is that it analyses the causes of the decrease in RGR due to water stress in eight species comprising four different growth forms. The results obtained strongly suggest that the influences of NAR, LAR, LMR and SLA on the decrease of RGR under drought depend on the species and growth form.

Changes in RGR with seedling age

In all the species analysed, the highest increase in total biomass occurred between H2 and H3 (Fig. 1). The change of RGR with increase in age, however, depended on growth form (Table 2). In those growth forms that invested more biomass in support structure, i.e. in perennial and semi-deciduous shrubs, RGRp decreased slowly with increase in age. RGRp of the perennial woody shrubs still increased even at the third harvest. On the other hand, growth forms of smaller adult size, i.e. perennial herbs and woody perennial plants, had the highest RGRp at early stages of development, and this

decreased strongly during plant development. This may be due to the different growth pattern of the species analysed: *Beta* and *Limonium* species developed a high LA at early stages and hence self-shading appeared at relatively early developmental stage. By contrast, in shrubs LA development was slower, and self-shading was not significant at the third harvest. This is well related to NARp changes by a strong positive relationship between RGRp and NARp within each species with plant age, suggesting the physiological factor as the main component determining RGRp changes, in accordance with Antúnez et al. (2001). Reich (1998) hypothesised that differences in growth between species with short and long leaf life-span tend to disappear over time due to differences in the time necessary to develop a full canopy. In our case, *Beta* and, to a lesser extent, *Limonium* species, were able to develop their full canopy at the end of the experiment. On the other hand, shrubby species invest more in support structures from the early stages onwards, and thus their full canopy takes more time to develop an efficient structure to capture incident light. Regarding the morphological traits, in all plants an increase in age resulted in increased biomass allocation to roots (increased RMR), decreased biomass allocation to leaves (decreased LMR), increased leaf thickness (decreased SLA) and, as a consequence, decreased LAR.

Water stress decreased the overall growth of all species included in this study (Table 2). In all species, total biomass of water stressed at the end of the experiment
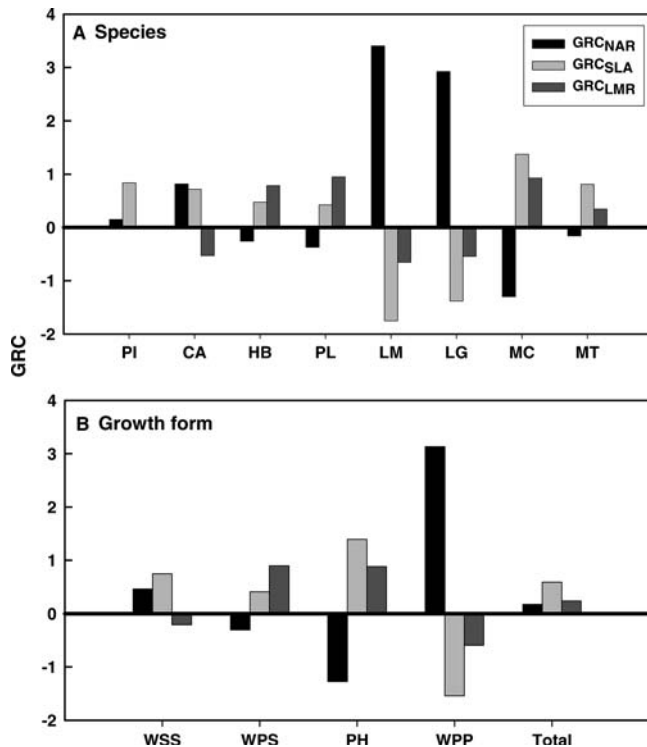
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Fig. 4 Growth relative components (*GRC*) for each of the species studied (**a**), growth form and pooling all species together (**b**). Woody hemi-deciduous shrubs (*WHS*), woody perennial shrubs (*WPS*), perennial herbs (*PH*) and woody perennial plants (*WPP*). Species codes as in Table 1. Calculations were done from the average values for each species shown in Table 2

was between 30% and 60% of irrigated plants, although the percent reduction tended to be lower in perennial species than in the other groups (Fig. 1). All species decreased LA under water limited conditions, which has been related to the ability of species to tolerate and acclimate to water stress by morphogenetic plastic responses (Kozlowski et al. 1991). However, three of the species analysed, CA, LM and LG, did not decrease the biomass allocation to leaves in relation to the total biomass (LMR).

Dependence of RGRt on morphological
and physiological components

The same trend in growth-form dependence observed in the developmental changes of RGRp and NARp was observed for RGRt: RGRt decreased from perennial herbs to woody perennial shrubs. Attending to the positive relationships between SLA and LMR with RGRt (Fig. 3c, d), this is due to a lower LAR or to a larger investment in supporting structures (Konings 1989) (Fig. 3b).

The strong positive relationship found in this study between RGRt and SLA supports most of the studies available from the literature, which consider SLA as the prime factor associated with interspecific variation in

RGRt (Lambers and Poorter 1992; Wright and Westoby 1999). Thus, a quantitative grouping of the different growth forms may be considered along the regression line between these parameters: the slow-growing species (i.e. woody perennial shrubs) had the lowest SLA, and the fast-growing perennial herbs the highest, while woody hemi-deciduous shrubs appeared intermediate (Fig. 3d). The growth form that lied more far from the regression line was that of woody perennial plants. A lower SLA is mainly due to a higher leaf mass density (Castro-Díez et al. 2000), and it has been shown to be inversely proportional to the internal gas conductance of leaves (g_i) (Syversten et al. 1995), and thus to the concentration of CO_2 in the chloroplast. This would imply that if, for given SLA, *Limonium* species have a higher RGRt, better carboxylation efficiency (i.e. photosynthetic gain per unit carbon available in the chloroplast) is likely found in *Limonium* than in the other species. This would be in accordance with the highest Rubisco specificity factor found in *Limonium* among C_3 species (Galmés et al. 2005).

The relationship between RGRt and the other morphological component, LMR was also positive (Fig. 3c). As a consequence, LAR exerted a positive influence on RGRt ($r = 0.726$, $P < 0.001$) (Fig. 3b). However, the lack of a positive relationship between RGRt and NARt (Fig. 3a) is in accordance with other authors (Dijkstra and Lambers 1989; Khurana and Singh 2000; Wright and Westoby 1999). Some possible explanations have been offered for this interrelation. First, Lambers and Poorter (1992) attributed this discrepancy to the resulting self-shading in plants with a high LAR. Secondly, since NARt is a complex function of photosynthesis, respiration and partitioning of biomass to photosynthetic and non-photosynthetic components (Lambers et al. 1989), fast respiration rates decrease NARt. Finally, Wright and Westoby (2000) concluded that the opposite trends in the leaf nitrogen productivity and the leaf nitrogen concentration with RGR explain the lack of relationship between NAR and RGR. Regarding the first hypothesis, species with lower NAR had the highest LAR ($r = -0.814$, $P < 0.001$) and self-shading events clearly appear in *Beta* and *Limonium* species. Moreover, when plotting RGRp versus NARp considering all species and all harvests a positive relationship between these parameters was obtained ($r = 0.532$, $P < 0.05$), suggesting that NAR determined plant growth at early stages of development when self-shading effects still did not influence photosynthesis. Although leaf dark respiration is just a part of total plant respiration, the second hypothesis cannot be excluded. For instance, species with higher RGRt and higher LAR have a lower proportion of daily carbon gain used in respiration (Poorter et al. 1990; Reich et al. 1998). The high proportion of biomass allocation to leaves, especially in the perennial herbs, must be at least considered as somewhat indicative of the high whole plant respiration rates of these plants. This weak negative relationship between RGRt and NARt when comparing different growth

forms, however, turns into a high positive interaction between RGRp and NARp when considering all harvests within each species. This suggests that within a single species the physiological component becomes the main parameter determining RGRp. A negative relationship when pooling all the species comes, therefore, from compensatory responses of morphological components of RGRt.

Components of the decreased RGRt under water-limited conditions

Pooling all species, the decrease in RGRt is mainly explained by decreases in SLA (Fig. 4b), in contrast with the results of Poorter and Nagel (2000), who found in 15 observations from literature that decreases in SLA and especially in NAR were the causes of changes in RGRt by water stress. However, they mainly considered grasses and only one of the species, *Sinapis alba*, occurs in the Mediterranean climate.

The relative contribution of each of the underlying growth parameters to the decrease of RGRt caused by water deficit strongly depended on the species and growth forms (Fig. 4). As hypothesised, the fast growing perennial herbs, which present a rapid leaf turnover, decreased their RGRt under water stress mainly due to morphological adjustments, while NARt was indeed increased possibly due to a strong reduction in self-shading (Lambers and Poorter 1992). Woody perennials, however, showed two distinct patterns: in woody perennial shrubs the decrease of RGRt under water stress was associated with morphological adjustments (decreased SLA and, especially, LMR), while in woody perennial plants it was associated with physiological adjustments (decreased NARt).

We had hypothesised that morphological adjustments may be expected in woody perennials only at early stages of development, and this seems certainly the case in shrubs. These achieved a 30-cm height at the end of the experiment, while their adult size is typically up to 2 m (*H. balearicum*) and 5 m (*P. lentiscus*). Therefore, they can be considered being at early stages of development during the entire experiment, and their response to water stress fits the hypothesised behaviour. In fact, a well known typical adaptation of Mediterranean woody perennials to drought is to increase the allocation of a greater biomass below than above ground, and to decrease the evaporating surface (Ludlow 1989). In this study, woody perennial shrubs decreased RGRt mainly because of a decrease in biomass allocation to leaves (Fig. 4b).

By contrast, woody perennial plants decreased their RGRt under water stress mostly due to a strong adjustment of NARt, a response that had been hypothesised mostly for adult woody perennial plants. In fact, because these plants achieve a very low height when adult (less than 20 cm), the studied plants at the end of the experiment were very close to their adult size.

However, the great contribution of decreases in NARt to the decrease in RGRt in woody perennial plants may be attributed to the observed increase in LAR, which in turn may enhance the effects of drought on photosynthesis and respiration. In conditions where carbon gain is low, as in water-limited conditions, respiratory carbon use is proportionally a much greater fraction of carbon gain, and thus plays a larger role in determining NAR and RGR (Flexas et al. 2005). For these species, in their natural habitats, the typical response of increasing allocation to roots (Ludlow 1989) may not be of adaptive value, since they inhabit over rocky surfaces with no easy access to deeper water or with access only to marine water intrusions.

Finally, as a group woody semi-deciduous shrubs showed an intermediate response, reducing RGRt under drought through a combination of morphological (decreased SLA) and, to a lesser extent, physiological (decreased NARt) adjustments (Fig. 4b). However, the two species belonging to this group presented significantly different patterns. In PI only decreases in SLA are responsible for decreased RGRt under water stress, while in CA decreased NARt contributed to a similar extent than decreases in SLA (Fig. 4a). These results suggest that the importance of several morphologic and physiological adjustments of RGRt to water stress may depend not only on the growth form but also, in some cases, on the species within a given growth form. These species are known to lose part of their foliage in the dry season (Flexas et al. 2003). In the present experiment, they certainly decreased LA strongly under water stress (Table 2), although this was more likely due to a reduction in leaf appearance. Nevertheless, these changes in the biomass allocation to leaves did not correlate significantly with the observed decreases in RGRt, which was mostly dependent on decreased SLA. These species are indeed known to produce leaves with a higher leaf mass per unit area under drought conditions (Margaris 1981).

In conclusion, the present paper showed that the influences of NAR, LMR and SLA on the decrease of RGR imposed by drought strongly depend on the species and growth forms. This might reflect differences in response and adaptation to environmental constraints.

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